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The competence of Pituitaries and Limb Regeneration during Metamorphosis of *Triturus (Diemyctilus) Viridescens*[1]

by

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With 7 figures

A la mémoire de notre Maître, Emile Guyénot, ce modeste travail est dédié en témoignage de gratitude et d'admiration.

Tant au début qu'à la fin de sa carrière, il a guidé nos premiers pas dans la recherche scientifique et nous a entraînés dans le monde merveilleux de la régénération. Par ses qualités de chercheur, sa vision précise du but à atteindre, sa méthode rigoureuse, il a été un exemple qui nous inspire continuellement et pour lequel notre reconnaissance est infinie.

INTRODUCTION

There are many instances of ontogenetic evolution of endocrine activities, to which Willier (1955) has so forcefully attracted attention. A striking illustration of gradual emergence of specific functions closely associated with visible ontogenetic manifestations are the successive phases in the metamorphic transformations of amphibia. Amphibian metamorphosis is clearly hormonally con-

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trolled (see general articles by Allen, 1938 and Etkin, 1955) and the early and overwhelming influence of the pituitary in anuran metamorphosis became a fact ever since Allen (1916) and Smith (1916) had demonstrated that extirpation of pituitary rudiments in frog embryos permitted the preservation of a larval status in maintaining these animals " indefinitely " at the tadpole stage.

The similar experiments by Blount (1932 and 1935) have confirmed the above results for urodele and Schotté (1926b) has succeeded in supressing metamorphosis in young larvae of Triturus for up to 22 months after hypophysectomy. But it is only recently that the more complex aspects of metamorphosis in urodele—such as the water drive in the Eastern American newt—have been approached and we owe to Reinke & Chadwick (1939), Chadwick (1940a, 1940b) and especially to Grant & Grant (1956, 1958) and Grant (1961) convincing experimental evidence. From all these researches the salient fact of the overwhelming role of the pituitary in the morphological and physiological transformations during the newt's ontogeny becomes manifest.

The importance of the pituitary's role in metamorphosis parallels that of the relevance of the pituitary in regeneration, as has been pointed out repeatedly in the past and more recently by Schotté (1961). It is indeed a fact that the gradual extinction of regenerative processes in limbs of anuran tadpoles is quasi epiphenomenal with the latter's metamorphosis. Equally impressive is the considerable decrescence in regenerative powers after metamorphosis of earth salamanders (*Salamandra* and *Amblystoma*) and the not inconsiderable lessening in the rates of regenerative processes after metamorphosis even in newts.

The congruency of pituitary action in urodele ontogenetic development with its role in regenerative processes becomes particularly striking when one considers the remarkable fact that before metamorphosis *Triturus* larvae regenerate their limbs in absence of the pituitary while the adult newt requires the presence of that gland for regeneration, inasmuch as after hypophysectomy amputated limbs of the latter do not regenerate (Schotté, 1926a) [1]. This diametrically opposite dependence of one process toward one

[1] In addition to the preliminary experiments of Schotté 1926, an extensive series of experiments on the influence of hormones in larvae of urodele have been performed at this laboratory (Richmond Mayo-Smith, 1946 Honors

and the same factor suggests two possible explanations: either the cellular nature of the larval limbs might have undergone at metamorphosis peculiar changes which radically modify the hormonal exigencies of these organs for regeneration, or one could hypothesize that the nature of the hormonal factor, namely the pituitary, has been substantially transformed. It is indeed enticing to speculate that during the decisive phases in the ontogenetic evolution of a gill-bearing water larva over a transitional land phase and finally to a completely adapted water newt, the pituitary undergoes a parallel metamorphosis on its own. Support for the more likely second interpretation comes from cytological studies by Atwell (1921), Copeland (1943), Dent (1961), Kent (1945) and Pasteels (1957) which all concur in showing that the cytological constituents of the anterior pituitary change in complexity during metamorphosis. If the changed ecological conditions are actually associated with the cytological alterations a parallel transformation in functionality of the pituitary during metamorphosis suggests itself.

The above mentioned researches had shown that the interdependence patterns between pituitary and regeneration change abruptly from larvae to adults, but nothing is known of the role of the pituitary upon regenerative processes during metamorphosis. The morphologically and particularly ecologically distinct stages of metamorphosis occurring in the Eastern American newt, *Triturus (Diemyctilus) viridescens* offer an uniquely suitable opportunity for an investigation to determine whether the morphologically discrete metamorphic stages of *Triturus* (the two " eft " forms we are describing) exhibit during the prolonged metamorphosis of the American newt a parallel ontogenetic physiogenesis in their pituitaries. By appropriate methods—hypophysectomy—changes in the pituitary's role as a hormonal determinant in limb regeneration of the metamorphic stages have been investigated. Moreover, in order to establish the nature of ontogenetic evolution of the eft's pituitaries appropriate transplantations between individuals of

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Thesis, Amherst College; Betty Bruening, 1958 M. A. Thesis, Amherst College). Some of the latter experiments were reported in Schotté 1961. In addition a new experimental research on the competence of the larval pituitary and its role in the regeneration of limbs in larvae and in adult urodele (Schotté, Bruening and Droin) is in the process of publication.

different ontogenetic ages permitted to assay the competence of metamorphic pituitaries as regulators of regenerative processes in general.

MATERIALS AND METHODS

All the experiments were performed upon postmetamorphic and upon adult *Triturus (Diemyctilus) viridescens* from Western Massachusetts.

The *Brown efts* (Fig. 1, hors texte) were all laboratory metamorphosed specimens which were caught as late gill-bearing swimming larvae from local ponds and which metamorphosed within a week or two after capture. They measured at the time of their first metamorphosis from 32 to 40 mm and they stayed with their typical " brown " pigmentation for a minimum of several weeks, but not longer than two months. They are difficult to feed in captivity and are not very resistant to trauma, hence their mortality after hypophysectomy was very high (up to 75% of the cases died within a few days after the operation).

The *Red efts* (Fig. 2, hors texte) are easily secured after a rain in woods adjacent to ponds and they varied in sizes from 40 to 75 mm when captured. They are much sturdier and resistant to trauma than the younger brown efts. When collected and operated upon only their sizes gave any indication as to whether they were in the second or in the third year after their first metamorphosis.

Fig. 1

Color photograph of the " Brown " eft stage after the first metamorphosis of *Triturus (Diemyctilus) viridescens.* This specimen was caught at the stage of a late swimming larva on July 26th in a pond of Western Massachusetts and it measured 41 mm when it left the water ten days later. The gills have completely regressed and the coloration of the integument at this stage is distinctly olive-brown in contrast to the lighter and greener color of the adult water form. The bilaterally situated but not necessarily symetrical orange-purple spots of the adult are present, but they are of a much lighter (yellowish) hue. Noticeable is the round tail following loss of the tail fins. (Normal size).

Fig. 2

Color photograph of a " Red " eft, the second terrestrial premetamorphic stage of *Triturus viridescens*, aged at least one year after its first metamorphosis. This specimen measured 48 mm and it was selected at random among a group of red efts measuring from 42 to 67 mm (average 61 mm), captured in nature on July 12, 1963. Notice the distinctly red-orange color of the skin and the presence of the much brighter " adult " orange-purple pigment spots.

Fig. 1

Fig. 2

The *hypophysectomies* in both types of efts and in adult newts were routinely performed by the usual method through the sphenoid of the mouth. When indicated, freshly extracted pituitaries were implanted either orthotopically within the sella turcica or heterotopically within the well vascularized subdermal tissues of the lower jaw. No difficulties were encountered in regard to survival and subsequent recovery of the transplanted hypophyses from efts into adult newts.

In view of the special nature of this research centering around the functionality of transplanted efts pituitaries into adults two prerequisites were required to ascertain the validity of the data: Firstly, it was necessary to verify that no remnants of supposedly completely extirpated hypophyses of adult newts were influencing results (a condition which might lead to erroneous interpretations, as shown by Hall and Schotté 1951); for that reason, whenever adult hypophysectomized newts were involved, their cranial cavity was carefully examined on serial sections and searched for remnants possibly resulting from faulty surgery. Secondly, whenever positive as against negative results were obtained from transplanted pituitaries from the two types of efts under investigation it was imperious to establish by adequate histological investigation whether the transplanted tissues had remained functional ex situ. When, for instance, a pituitary from a particular type of eft was shown not to be competent to replace the missing adenohypophysis of an adult this result was accepted only after ascertaining that the transplanted eft's gland was actually surviving within its new host, that it was well vascularized and that its cellular constituents had maintained a cytological aspect compatible with functionality [1].

EXPERIMENTAL

Two distinct series of operations with separate purposes in mind were performed: In a first series brown and red efts were hypophysectomized and their forelimbs amputated to ascertain

[1] The scope of this research does not include detailed observations in regard to general biological effects of the transplantations of pituitaries from both brown and from red efts upon hypophysectomized adult newts. Suffice it to state that they survived well within the tissues of the adult and that they remained functional ex situ for well over a month.

whether, in respect to hormonal requirements for regeneration, the pituitaries of these two postmetamorphic efts functioned according to the larval or to the adult urodele type. In a second series of operations the competence for regulating adult regeneration of the pituitaries from these two eft types was tested by transplanting them into hypophysectomized and amputated adult newts.

Forelimb Regeneration in Hypophysectomized Brown and in Red Efts

1. *Brown eft regeneration after hypophysectomy.* A total of 51 individuals obtained as described above were hypophysectomized within the first week after leaving the water. Of these only 15 cases survived long enough for adequate study. (Series A, necessary data and results on Table 1, figures 1 and 3 with detailed information.)

The data show that regeneration of the forelimbs was observed in every one of the surviving cases; furthermore, its normalcy and extent depended upon the amputation age of the eft's forelimbs rather than upon the histologically verified absence (13 cases) or upon partial presence of the brown efts' pituitaries (2 cases). Evidence to the behavior of the former group is given in figure 3, a microphotograph from a forelimb of a freshly metamorphosed brown eft with histologically verified total hypophysectomy. It is essential to state that the two efts in which remnants of an incompletely extirpated pituitary were found within the sella turcica did not regenerate any better or any faster than the other thirteen cases. Presence or absence of the pituitary simply does not make any difference in regeneration, a fact which was amply confirmed from numerous control and other experiments performed upon brown efts at this laboratory.

This shows that after loss of gills and the acquisition of many other morphological and physiological features following the first metamorphosis the brown land efts of Triturus regenerate their limbs in a manner similar to the one prevailing in larvae of urodele (Schotté 1926a, 1961)—that is in absence of their pituitaries.

2. *Forelimb regeneration in hypophysectomized Red efts.* All individuals were caught at random in nature, therefore of indeterminate age. However, since their capture coincided with that of advanced but still gill-bearing larvae which were the source of the

Fig. 3

Photomicrograph of section from left forelimb of case HE_{29}, a freshly metamorphosed hypophysectomized brown eft. The limb was fixed 24 days after hypophysectomy, 22 days after amputation. The quasi larval nature of this limb is recognizable by the aspects of the epidermis and the dermal structures, the thinness of muscle bundles and the still cartilaginous nature of the long forearm bones, both exhibiting only tenuous laminae of a periosteal bone collar. Distad to the amputation area, marked by the cut surfaces of radius and ulna, full regeneration is in progress. Comparison with limbs from unoperated eft controls of the same amputation age suggests that, in the early brown eft stage, normal regenerative processes are not affected by the removal of the hypophysis. (X 120).

" brown " efts of the previous series it is clear that they had metamorphosed during the previous year. The smallest red efts used (over 40 mm) were therefore at least one year old. The larger ones (those measuring over 60 mm in length) must have lived on land for over two or perhaps even three years. In addition, many of the efts of this series were operated only after an additional sojourn of several months at the laboratory, without however undergoing their second metamorphosis. (Series B, Table 1, second horizontal row, also figures 2 and 4; a comparison of figures 3 and 4 is instructive.)

Table 1

*Effects of hypophysectomies upon forelimb at two stages of efts: Series A. Brown efts, laboratory metamorphosed, and measuring from 34 to 39 mm. Series B. Older, Red efts, captured in nature and measuring from 45 to 57 mm. (Cases marked with * indicate conditions rendering histological verifications impossible.)*

Number of Cases	Amputation Age at Fixation (Days)	Results from histological examination: Limb Regeneration: Present	Limb Regeneration: Absent	Pituitary Remnants: Present	Pituitary Remnants: Absent
Series A. Regeneration in hypophysectomized brown efts					
2	14	2	—	—	2
2	15	2	—	—	2
2	17	2	—	—	2
2	20	2	—	—	2
7	22	7	—	2	5
15		15	—	2	13
Series B. Regeneration in hypophysectomized red efts					
2	14	—	2	—	2
2	16	—	2	—	2
3	18	—	3	—	3
1	19	—	1	—	1
2	20	—	2	—	2
5	21	1	4	1	4
1	22	—	1	—	1
3	24	3	—	1 (2 *)	—
19		4	15	4	15

The results from nineteen usable survivors of this group (Series B, Table 1) show that no regeneration occurred in fifteen of the nineteen cases studied. The section of a forelimb from such a red eft represented on figure 4 (Case EHC_{18}, similar to the other fourteen cases), exhibits a pattern of histological features characteristic for nonregeneration, such as obtained from limbs of adult hypophysectomized newts, fully described by Hall and Schotté, 1951. (Compare also with figure 5.)

The remaining four cases, although not exhibiting upon gross examination any visible regeneration, showed, on slides, numerous blastematous cells. In no case, however, did this accumulation of

dedifferentiated cells develop into the familiar aspect of a normal blastema. For two cases this aberrant behavior was explained by pituitary remnants found within the cranial cavity, thus obviously resulting from incomplete hypophysectomies. In the other two

FIG. 4

Photomicrograph of the left forelimb of an hypophysectomized red eft. (Case EHC_{18} amputated through the radio-ulna and fixed at 21 days amputation age.) The shredded aspect of the outer layers of the epithelium is similar to the one observed and described in hypophysectomized adult *Triturus*. The limits of the amputation surface are indicated by the dermal and subdermal tissues and by the presence of skin glands characteristic for normal skin. The wound epidermis is typical, because of its apical cap, for regenerating limbs, if it were not for a precocious infiltration of dermal elements. There are indications of some dedifferentiative activities within the distal ends of the cut muscle fibers, within the periosteum and around the shattered bones. The lack of any sizeable accumulation of blastematous elements, however, combined with the presence of adult fibroblasts adjacent to the wound epidermis are typical for a nonregenerating limb. (X 120).

cases, however, such remnants were not detectable because of an unfortunate mishaps in histological procedures. In view of the cumulative evidence gained at this laboratory it can safely be deduced that the abortive regeneration obtained in these two latter somewhat mangled cases can safely be attributed to some post-operative pituitary debris surviving within the brain cavity.

The experiments of Series A and B thus provide indisputable evidence that, in the ontogeny of the efts of Triturus, two phases

in the physiogenesis of pituitary action upon regeneration are detectable: (a) immediately after metamorphosis and for sometime later in the *brown eft* stage regenerative processes do occur in absence of the pituitary: (b) in the *red eft*, on the contrary, the presence of the hypophysis becomes just as mandatory for regeneration as it is in the case of adult newts.

Whether the pituitaries of these two distinct ontogenetic stages of the efts are sufficiently evolved to substitute for the adenohypophysis of an adult newt cannot be safely predicted. The following experiments were designed to test the competence of the brown and the red eft's pituitaries to act as hormonal determinants for regeneration.

Regenerative Patterns in Forelimbs of Hypophysectomized Adult Newts After Implantation of Pituitaries From Brown and From Red Efts

In the following experiments the host adult water newts were routinely hypophysectomized by one of us, while the other coauthor removed a pituitary from a brown or from a red eft. Without any further delay the freshly extracted eft pituitaries were implanted into the adult host either orthotopically or heterotopically, as will be specified below. The amputation of one forelimb (through mid-humerus) of the graft bearing hypophysectomized newt followed two days later.

1. *Effects of substitution of the adult newt's adenohypophysis by a brown eft's pituitary.* The efts serving as pituitary donors came from individuals having undergone their first metamorphosis at the laboratory not more than a week or ten days before. In this series the pituitaries were implanted orthotopically, into the sella turcica of the newt.

The data dealing with the sizes of the donor efts, with observations in regard to the newt's limb regeneration, with the survival of the transplants and finally with the search on slides of the newt's brain cavities in regard to possible pituitary remnants are given in Table 2 (Series C). In one case only did the histological examination fail to reveal any trace of the transplanted eft's pituitary, the other twelve newts exhibiting transplants in excellent condition.

In regard to regeneration, one limb only among the thirteen examined showed regeneration and this limb belonged to a newt in which, on sections, there were present identifiable remnants of its

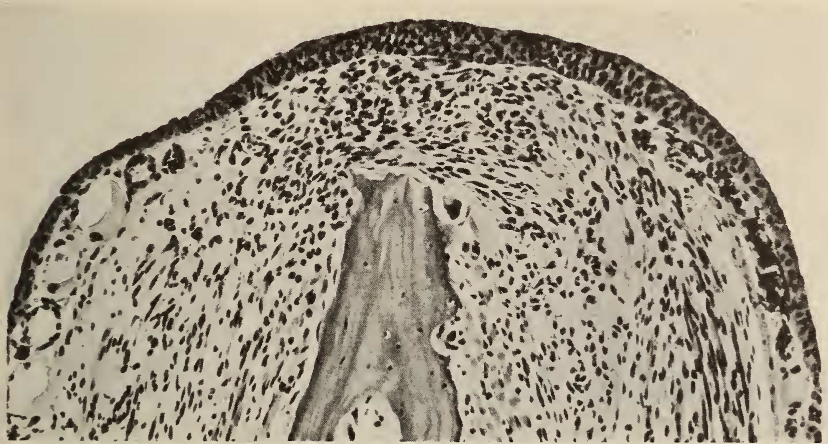

FIG. 5

Photomicrograph of a section of the left limb of an adult *Triturus* (Case $EHTA_{23}$ having received a pituitary transplant from a freshly metamorphosed brown eft and fixed at 21 days amputation age.) The limits of the amputation area are discernible at the left ventral side by presence of the skin glands and, at the right dorsal side, by layers of chromatophores. The amputation surface is shown to be reduced to about one third of the width of the limb, the constriction being supported by the inward orientation of tendons, muscle fibers and fibroblastic bundles, all converging from the lateral aspect to the center of the amputation area. In that central area however the epidermis does not exhibit the typical aspects of an apical cap invariably found in a regenerating limb. Beneath the wound epidermis there are dermal elements amidst which a disarray of blastematous cells may be observed, small in number and intersperced with differentiated fibroblastic elements. Also, the ground substance presents, on slides, the heterochromatic aspect of an adult loose connective tissue, not the smooth uniformity one encounters in a blastema. Finally, the cap-like mass of fibroblasts athwart the tip of the radius (in addition to negligible dedifferentiative activity observable only within the periosteum of this bone) are further contributory observations supporting the diagnosis of a type of wound healing which renders further regenerative processes impossible. (X 120).

own adenohypophysis. The limbs of the other twelve newts. in spite of the presence, except in one case, of surviving brown eft pituitary transplants, exhibited the patterns of nonregeneration characteristic for limbs of properly hypophysectomized newts.

The inefficacity of a brown eft's pituitary to act as a replacement for the adult newt's adenohypophysis is demonstrated by a section of a limb (Case $EHTA_{23}$, fig. 5) randomly selected among twelve entirely similar cases of this series C.

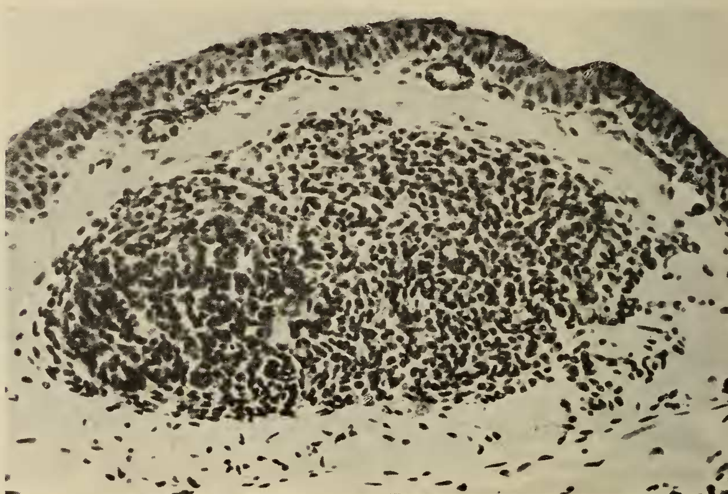

Fig. 6

Photomicrograph of section from jaw of adult *Triturus* (Case $EHTJ_{13}$) with a pituitary from a red eft 27 days after transplantation. The aspect of nuclei of the transplant is healthy and examination with the highest objectives does not reveal any nuclear deterioration. The likelihood of functionality of the eft's hypophysis heterotopically transplanted is indicated by its cytological aspect and by its ample vascularization, numerous capillaries being filled with erythrocytes. (X 170).

2. *Effects upon forelimb regeneration of transplanted red eft pituitaries in hypophysectomized adult Triturus.* In 51 cases pituitaries from red efts of the second or third year terrestrial phase were implanted, immediately following the adult newt's hypophysectomies: (a) either orthotopically into the sella turcica (34 cases) or (b) heterotopically into a skin pocket of the well vascularized lower jaw of freshly hypophysectomized newts (17 cases).

The results from these operations, summarized for Series D in Table 2 show that, *in regard to the success of the hypophysectomies and the transplantations* (a) the removal of the newt's adenohypophysis was faulty in two cases only; (b) that in the remaining

49 cases, in verified absence of any adult adenohypophysis, the transplanted red eft pituitaries were histologically recovered in the form of diagnostically suitable cellular masses within the tissues of adult hosts, no matter how long the experiment. The modus

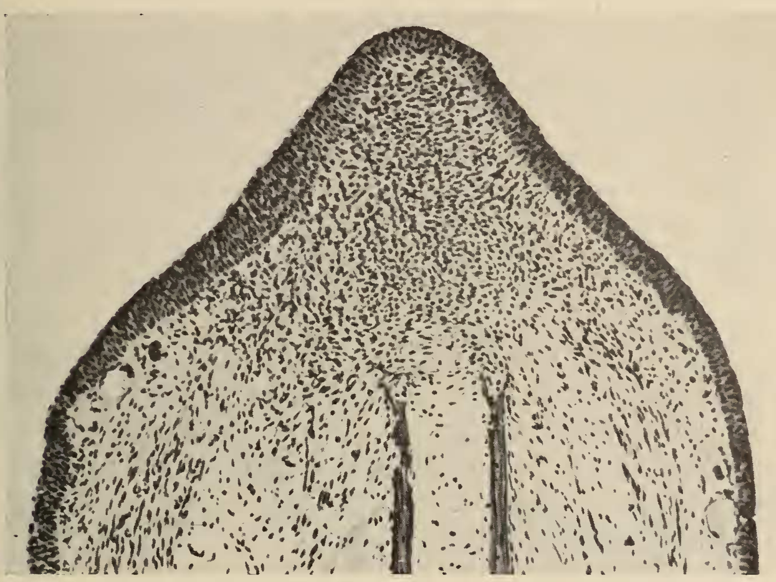

FIG. 7

Photomicrograph of left forelimb (fixed at 27 days amputation age) from an hypophysectomized adult newt (Case $EHTJ_{13}$) with second year red eft pituitary heterotopically transplanted into its lower jaw (see fig. 6). The demarcation between old limb tissues and regenerate is indicated by the presence of skin glands on either side of the limb and by the thick epidermal cap within the regenerating area. There is no dermal lining along the entire protruding cone-shaped formation and extensive mitotic activity within the epidermis is conspicuous on sections. The entire regeneration cone is densely populated with blastematous cells and cephalad to the cut bone there are indications of the onset of morphogenetic processes; this constitutes an aspect of regeneration typical and normal for this amputation age. (X 75).

operandi in substituting the red eft's pituitaries for those of the adults makes no difference in the histological aspects of the former which appear the same, independently of their implantation site.

In fig. 6 a photomicrograph from a red eft's pituitary, fixed twenty-seven days after its implantation to the lower jaw is represented for Case $EHTJ_{13}$, (the same newt from which a section of its limb is shown on fig. 7). The vascular supply around and whitin

the transplant (ascertained by presence of numerous host capillaries filled with erythrocytes) and the healthy aspect of the transplants nuclei provide suggestive evidence for the functionality of the eft's pituitary under these heterotopic conditions.

TABLE 2

*Effects of pituitary transplantations from freshly metamorphosed brown efts (Series C) and from the older terrestrial red efts (Series D) upon limb regeneration of hypophy sectomized adult Triturus. The eft pituitaries were implanted either orthotopically into the cranial sella turcica or heterotopically into the lower jaw of the adult hosts. Limb regeneration was ascertained by histological examination of every limb; histological scrutiny also permits diagnostic appraisal of survival and functionality of the transplanted eft's pituitary. (Cases with asterisk * refer to positive findings of the adult host's own pituitary; bracketed cases without asterisk refer to cases where no eft pituitary was found.)*

		Histological findings and verifications				
		Number of Newts	Amput. Age at Fixation	Limb Regeneration		Status of Transplant
				Reg. Pres.	Reg. Abs.	
Series C	Pituitaries from young postmetamorphic brown efts (29-35 mm) implanted into adult newts.	1	15 days	—	1	1 good
		3	18 days	—	2 (1)	2 (one missing)
		4	19 days	—	4	4 good
		5	21 days	1 *	4	5 good
	Totals	13		1	12	12 (1) good
Series D	Pituitaries from second year red efts (40-64 mm) implanted into adult newts.	4	17-20 days	4	—	4 good
		25	21 days	23 (2*)	—	23 (2*) good
		22	24-34 days	22	—	22 good
	Totals	51		49 + (2*)	—	51 good

In regard to regeneration of limbs: (a) the two cases (among 51) of positive regeneration coinciding with histologically detectable remnants of the hosts adenohypophysis must be discarded on grounds of faulty surgery; (b) in the remaining 49/51 cases regeneration was observed in every limb. This result cannot be attributed

to faulty surgery, since the sella turcica was found devoid of pituitary remnants and under those conditions countless experimentation has proven that adult newts do not regenerate their limbs when hypophysectomized. Regeneration then, in the forty-nine cases, in which at the end of the experiment, the transplants were recovered in situ and ex situ, can be attributed only to the red eft pituitaries found within the hypophysectomized newts.

Among these numerous cases a section from the limb of the above mentioned newt $EHTJ_{13}$ has been selected for illustration, (fig. 7) becauses it provides especially convincing evidence: it has regenerated in a newt, the sella turcica of which was clearly devoid of suspicious adult pituitary tissue remnants; in addition, the survival and probable functionality of the eft's pituitary placed into the jaw of the adult newt (fig. 6) is suggested by its cytological appearance maintained for as late as 27 days after heterotopic transplantation.

These two preceding series have produced distinctly opposite results: (1) Pituitaries from brown efts transplanted into a hypophysectomized newt do not substitute for the adult's missing adenohypophysis, since limbs from such newts do not regenerate. (2) In striking contrast to the above, transplanted red eft pituitaries exhibit full competence to substitute for the newt's missing adenohypophysis: in every case in which the two prerequisites of the experiment, namely faultless removal of an adult adenohypophysis and survival of an orthotopically or heterotopically transplanted red eft pituitary were satisfied normal regeneration ensued.

SUMMARY AND CONCLUSIONS

The remarkable double metamorphosis which occurs in *Triturus viridescens* during the change from a gill-bearing water larva over two land stages to the final adult water form has been previously proven to be involved with this animal's pituitary. That the morphological and cytological changes of metamorphosis are not exclusively determined by the pituitary is surely a fact (Kollros 1961), but this research has shown that in *T. viridescens* the dramatic hormonal involvements of metamorphosis are reflected also in the processes of regeneration.

For these reasons, the question posed in the introduction as to whether after the first metamorphosis, the pituitary of the two successive eft forms would, in respect to regeneration, behave as does a pituitary in larval urodele was legitimate. The answer to that question could, however, be expected only from ad hoc experiments, since it was just as logical to assume that metamorphosis meant an ontogenetically parallel transformation of the larval pituitary into an adult type adenohypophysis, fully incorporated within the pituitary-adrenal system of the final water form. To these alternative solutions the foregoing experiments have provided nonequivocal answers.

The relative saliency of the problem permitted the use of some unsophisticated methods, entirely analogous to those used in the past, namely removal of the gland to be tested and examination of the effect of its removal upon regeneration. Another time-honored method consisted in the transplantation of the gland of still uncertain function to another animal after extirpation, prior to the transplantation, of the gland with already known function. The results from the application of these two simple methods were clear, but the burden of the research consisted in tedious and time consuming verifications without which the results were devoid of any demonstrative value. The following statements and conclusions seem to be amply supported by the new evidence obtained.

1. After completion of the first metamorphosis the pituitary of the first terrestrial form—the brown eft, does not influence the course of regeneration: *amputated limbs in hypophysectomized brown efts regenerate as do unoperated brown efts with their pituitaries intact.*

2. The orthotopic transplantation of pituitaries from brown efts into properly hypophysectomized adult newts does not modify the course of regenerative events, since hypophysectomized adult newts with surviving brown eft transplants do not regenerate their limbs.

3. It follows that *the pituitary of brown land efts is* entirely *analogous to a larval urodele pituitary:* as hormonal determinants of regenerative processes, both larval and brown eft pituitaries are incompetent.

4. Before completion of the final metamorphosis the pituitary of the second terrestrial phase—the red eft deeply influences the

course of regeneration, since faultlessly hypophysectomized red efts do not regenerate their limbs.

5. When transplanted orthotopically or heterotopically into properly hypophysectomized adult newts, red eft pituitaries are capable of functional survival and they are fully competent to substitute for the missing adenohypophysis, inasmuch as adult newts regenerate normally when red eft pituitary transplants are used.

6. It is concluded that *the pituitary of a red eft is at an ontogenetic stage analogous to that of an adult newt*; *it is*, at least *in regard to its role in regeneration, a true adenohypophysis.*

RÉSUMÉ

Le triton de l'est des Etats-Unis présente un cycle vital particulier. A partir des œufs, pondus au printemps, se développent des larves à branchies externes qui subissent, à la fin de l'été, une première métamorphose les transformant en « efts » bruns, stade terrestre de courte durée. Après quelques semaines, la pigmentation se modifie, l'animal prend une teinte orangée, devient un « eft » rouge, également terrestre, et vit ainsi pendant plusieurs années. Ce n'est qu'après une deuxième métamorphose que le triton prend sa forme adulte définitive, vert-olive, et retourne à la vie aquatique.

De nombreuses recherches ont montré, chez les larves d'urodèles, l'incompétence de l'hypophyse, comme facteur responsable de la régénération des membres. Cette étude a pour but de déterminer si l'hypophyse des deux stades terrestres, brun et rouge, fonctionne selon le type larvaire ou adulte. Les résultats montrent que a) les pattes des efts bruns régénèrent après hypophysectomie comme le font celles des larves; les hypophyses d'efts bruns transplantées chez des tritons adultes hypophysectomisés n'influencent en aucune façon la non-régénération des animaux ainsi traités, ce qui démontre que l'eft brun possède une hypophyse de type larvaire. b) Les efts rouges, par contre, ne peuvent régénérer leurs membres après hypophysectomie. D'autre part, les pattes des tritons adultes hypophysectomisés, normalement incapables de régénérer, peuvent le faire après transplantation d'hypophyses d'efts rouges à la place

de leur propre hypophyse; celles-ci sont donc capables de remplacer totalement les hypophyses adultes. Contrairement à l'hypophyse de l'eft brun, celle de l'eft rouge a atteint, avant la deuxième métamorphose, le stade ontogénétique d'une véritable hypophyse adulte, responsable des processus régénératifs.

A l'ontogénie morphologique du triton américain (larve aquatique, deux efts terrestres différents et triton mature aquatique) correspond une évolution ontogénétique de son appareil hypophysaire en tant que déterminant hormonal de la régénération.

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